KPNA2 protein expression in invasive breast carcinoma and matched peritumoral ductal carcinoma in situ

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ORIGINAL ARTICLE

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Keywords Breast cancer · DCIS · KPNA2 · Prognostic marker · Immunohistochemistry

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Introduction

Breast cancer is the most common malignant neoplasm of women in the Western world, with an estimated number of 178,480 new cases of breast cancer among US women in 2007 [4]. Recent diagnostic and therapeutic advances are encouraging, but at the same time, increasingly detailed prognostic information is required, which should exceed conventional prognostic parameters, i.e., age, nodal status, tumor size, tumor grade, tumor type, and receptor status. This condition seems to be met by several promising molecular markers like Kallikrein 5 [20], urokinase plasminogen activator and its inhibitor [9], tissue inhibitor of metalloproteinase 1 [10], Ep-CAM [17], osteopontin [12],

CD24 [8], SFRP1 [7], survivin [13, 16], and Karyopherin alpha 2 (KPNA2) [2, 15, 19].

Several gene expression profiling studies demonstrated overexpression of KPNA2, a member of the karyopherin alpha protein family, in breast cancer tissue [2, 15, 19]. Proteins of the karyopherin alpha family play a central role in nucleocytoplasmic transport. They act as an adaptor in the nuclear import of macromolecules by binding cargoproteins, which contain a classical nuclear localization signal, thereby linking them to the transport protein karyopherin beta. A strong nuclear KPNA2 immunoreactivity was observed in breast cancer cells compared to a weak or absent staining in normal breast tissue by Dahl et al. [2]. They also demonstrated an independent negative correlation between nuclear KPNA2 protein expression in the primary tumor and overall survival of breast cancer patients.

So far, no data on KPNA2 protein expression in preinvasive lesions of the breast are available. We, therefore, aimed to investigate the expression of KPNA2 immunohistochemically in a cohort of clinically characterized breast cancer samples and matched peritumoral ductal carcinoma in situ (DCIS) to verify the prognostic properties in invasive cancer and to focus on KPNA2 expression in adjacent DCIS.

Materials and methods

Patients

The study included 83 patients with breast cancer, diagnosed at the Institute of Pathology, University Hospital Charité, Berlin, between 1991 and 1997. Patient age at the time of diagnosis ranged from 30 to 80 with a mean of 58 years. Clinical follow-up data, including overall survival and disease-free survival, were available for all cases. The median observation time for overall survival was 78 months for patients still alive at the time of analysis (range 3–162 months). Fourteen patients (16.9%) died during follow-up, and 31 patients (37.3%) experienced disease progression, defined by either metastatic disease or local recurrence.

The selection of cases for this study was based on availability of tissues. Patients with systemic disease (pM1) at the time of diagnosis were excluded. Histological typing of tumors was carried out according to the criteria of the World Health Organization. Tumor stage was determined according to the guidelines of the UICC [14]. Tumors were graded according to Bloom and Richardson in the modification of Elston and Ellis [3]. Data regarding the estrogen receptor (ER) status, the expression of Her-2/neu (c-erbB2), and the proliferative fraction (Mib-1) were gathered from the archival pathology reports. The clinicopathological data of the cases are described in Table 1.

Table 1 Clinicopathological characteristics of primary breast carcinomas

Variable	No. (%)
Patient age	
<60 years	47 (56.6)
≥60 years	36 (43.4)
pT status	
pT1	51 (61.4)
pT2	24 (28.9)
pT3	6 (7.2)
pT4	2 (2.4)
pN status	
pN0	34 (41.0)
pN1	21 (25.3)
pN2	14 (16.9)
pN3	13 (15.7)
Histological grade	
G1	19 (22.9)
G2	40 (48.2)
G3	24 (28.9)
Histology	
Ductal	78 (94)
Lobular	5 (6)
Estrogen receptor	
Negative	19 (22.9)
Positive	55 (66.3)
Unknown	9 (10.8)
HER2 expression	
0/1+	45 (54.2)
2+/3+	19 (22.9)
Unknown	19 (22.9)

Immunohistochemistry

Immunohistochemistry (IHC) staining was performed using formalin-fixed, paraffin-embedded archival tissues. After sectioning, tissue sections were applied to slides, deparaffinized with xylene, and gradually rehydrated. Antigen retrieval was performed using a microwave oven (10 min at 250 W). The primary antibody anti-KPNA2 (goat polyclonal SC6917, Santa Cruz Biotechnology; dilution 1:200) was incubated at room temperature for 1 h. Detection was carried out using an avidin–biotin peroxidase method with 3,3′-diaminobenzidine as chromogen (ChemMate detection kit, DAKO, Glostrup, Denmark). Brief nuclear counterstaining with hematoxylin completed the procedure.

While normal testicular tissue served as a positive control for KPNA2 IHC, unspecific binding of the secondary antibody was excluded by omitting the primary antibody.

Evaluation

Evaluation of the immunohistochemical stainings was independently carried out by two pathologists who were

unaware of the outcome of the disease. Areas of invasive carcinoma, DCIS, and adjacent histologically benign breast tissue were considered separately. KNPA2 positivity was defined as strong nuclear staining in at least 10% of cells.

Statistical analysis

Data were analyzed using the software package SPSS, version 13.0. Spearman's rank correlation was calculated, and Fisher's exact test and χ^2 test were applied to evaluate the statistical significance of association between expression of KPNA2 and clinicopathological variables.

For univariate survival analysis, cumulative survival curves were calculated according to the Kaplan–Meier method. Differences in survival were assessed with the log-rank test. The impact of KPNA2 positivity, patient age, histologic tumor type, pT status, pN status, tumor grade, ER status, and cerbB2 status on overall survival time and disease-free survival time was analyzed.

Results

KPNA2 immunostaining showed a predominantly nuclear staining pattern, restricted to epithelial cells of normal breast glands, DCIS, and invasive breast cancer (Fig. 1). Additionally, a weak-to-moderate cytoplasmic staining was seen in some cases, but was not subjected to further analysis. Connective tissue cells, blood vessels, and inflammatory cells did not display KPNA2 immunoreactivity.

KPNA2 immunostaining in normal breast tissue

Normal breast tissue adjacent to tumor was present and evaluable in 78 (94%) patients. Nuclear staining was only seen in ten (12.0%) samples. As the stained nuclei never exceeded 1%, all samples were considered KPNA2 negative.

KPNA2 immunostaining in intraductal carcinoma

Seventy-five (90.4%) invasive breast cancer specimens contained peritumoral DCIS, of which 40 cases (53.3%) were of non-high grade and 35 (46.7%) were of high grade. Staining of KPNA2 in at least 10% of nuclei was detected in 16 samples (21.3%), whereas 59 samples (78.7%) were KPNA2 negative. High-grade DCIS displayed KPNA2 staining in at least 10% of nuclei significantly more frequently (n=12, 34.3%) than non-high-grade DCIS (n=4, 10.0%; p=0.013). According to this, 23 samples of high-grade DCIS (65.7%) and 36 samples of non-high-grade DCIS (90.0%) were KPNA2 negative. KPNA2 immunoreactivity in DCIS correlated with KPNA2 expression in adjacent invasive carcinomas (cc=0.725; p<0.001).

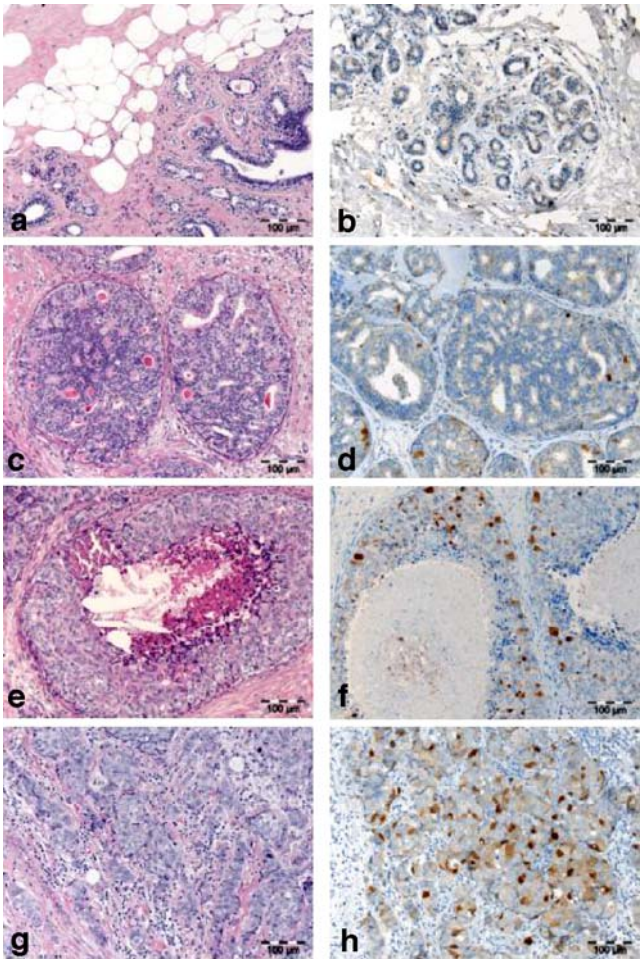

Fig. 1 Hematoxylin and eosin staining and KPNA2 immunohistochemistry. **a** Normal glandular breast tissue (HE). **b** Normal glandular breast tissue displaying luminal epithelium with only single KPNA2-positive nucleus and weak-to-moderate cytoplasmic staining. **c** Non-high-grade DCIS (HE). **d** Few KPNA2-positive nuclei and weak cytoplasmic staining in non-high-grade DCIS. **e** High-grade DCIS (HE). **f** High-grade DCIS with markedly increased nuclear KPNA2 staining. **g** Invasive ductal carcinoma (HE). **h** Strong nuclear KPNA2 staining and moderate cytoplasmic staining in invasive ductal carcinoma. **a**–**h** Original magnification ×200

KPNA2 immunostaining in invasive breast carcinomas

KPNA2-positive immunostaining was seen in 26 (31.3%) invasive carcinomas, the median percentage of positive nuclei being 15%, with a maximum of 40%. KPNA2 positivity of invasive carcinoma revealed a statistically significant correlation with higher pT status, nodal status, histological grade, and a negative ER status (Table 2).

KPNA2 immunoreactivity in invasive carcinomas and univariate survival analysis

Patients with KPNA2-positive breast carcinomas (≥10%) had an estimated mean disease-free survival time of 69 months (95% confidence interval, 47–92 months)

Table 2 Correlation of KPNA2 immunoreactivity in invasive breast cancer with conventional clinical or tumor parameters

KPNA2	DCIS	pT status	pN status	Grading	ER status	HER2
Correlation coefficient	0.725	0.321	0.390	0.470	−0.316	0.148
Significance (two-sided)	0.000	0.003	0.000	0.000	0.006	0.244
N	75	83	82	83	74	64

compared to 118 months (95% confidence interval, 100–135 months) in patients with negative KPNA2 staining (<10%; $p=0.007$; Fig. 2). For overall survival, no significant differences were noted (data not shown).

Discussion

Nucleocytoplasmic transport mechanisms have been the target of numerous studies for they are involved in many cellular processes, such as gene expression, cell-cycle progression, and signal transduction [1]. Regulating the subcellular distribution of macromolecules, nuclear transport mechanisms may also control their functions. There is increasing evidence that these mechanisms may contribute to malignant cell transformation, which makes their elements interesting targets for further evaluation [11]. One of these elements is KPNA2, an adaptor protein, which mediates the nuclear import of macromolecules with a classical nuclear localization signal. Linking a cargoprotein and Karyopherin beta, KPNA2 initiates the transport through the nuclear core complex. Thakur et al. [18] demonstrated that one of the proteins whose subcellular distribution may be influenced by KPNA2 is BRCA1, thereby connecting KPNA2 expression with breast carcinogenesis.

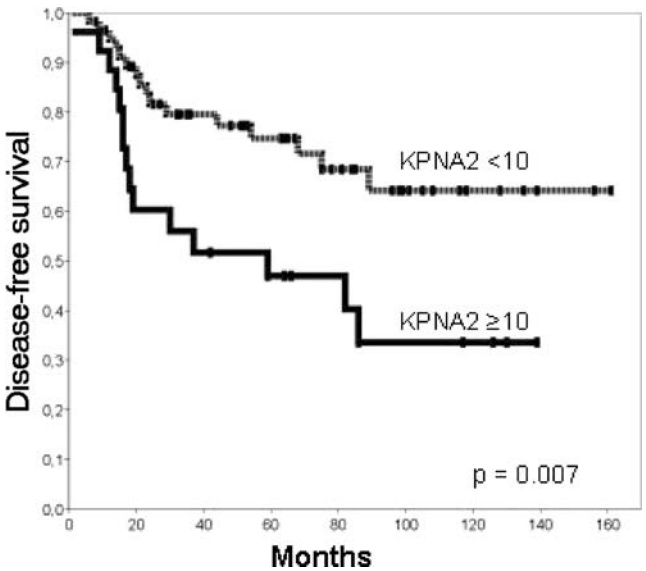

Fig. 2 Survival analysis. Kaplan–Meyer curves regarding disease-free survival of patients with KPNA2 expression in less than 10% of nuclei (*dashed line*) vs patients with KPNA2 expression in at least 10% of nuclei in invasive carcinomas (*bold line*)

Another cargoprotein of KPNA is TP53. Kim et al. [5] identified a truncated form of KPNA in a breast cancer cell line and showed that overexpression of this truncated form results in cytoplasmic accumulation of TP53, whereas overexpression of intact KPNA leads to a transactivation of TP53-responsive genes in the nucleus.

Several gene expression profiling studies reported overexpression of KPNA2 in breast cancer [2, 15, 19]. Sotiriou et al. [15] found an association of KPNA2 overexpression with higher histologic tumor grade, which matches our findings on protein level. Dahl et al. were the first to evaluate KPNA2 protein expression in breast cancer and observed a strong nuclear immunohistochemical staining in breast cancer cells compared to a weak or absent staining in normal breast tissues. They also demonstrated an independent negative association between KPNA2 expression in the primary tumor and overall survival in breast cancer patients which was particularly valid in the group of node-positive cases. Importantly, KPNA2 expression appears to be characteristic of the basal-like subtype of breast cancers, which has an immediate impact on adjuvant therapy planning [2].

The present study confirms our former results in an independent and well-characterized breast cancer cohort, using the cutoff value applied by Dahl et al. [2]. KPNA2 expression in at least 10% of nuclei was seen in 31% of invasive carcinomas, whereas none of the matched normal tissues was KPNA2 positive. Nuclear KPNA2 staining was significantly correlated with higher tumor stage, lymph node status, higher tumor grade, and negative ER status. Consistent with the results reported by Dahl et al., survival analysis revealed that patients with KPNA2-positive breast carcinomas had significantly shorter disease-free survival times, which clearly validates the prognostic value of KPNA2 in breast cancer.

Further investigation of KPNA2 expression in preinvasive lesions of the breast appears mandatory to establish the time point of KPNA2 upregulation in the multistep process of mammary carcinogenesis. To our knowledge, the present study includes the first description of KPNA2 protein expression in matched DCIS and invasive lesions of the breast. Staining of KPNA2 in at least 10% of nuclei was detected in 21% of patients with DCIS. KPNA2 immunoreactivity in DCIS and invasive carcinomas correlated significantly. The portion of KPNA2-positive cases increased successively, comparing adjacent benign tissues (0%), DCIS (21.3%), and invasive carcinomas (31%). This indicates, that upregulation of KPNA2 may be an early

event in carcinogenesis, which is also assumed by Klein et al. [6], who found KPNA2 upregulated early in a mouse model of breast cancer. This hypothesis is corroborated by the fact that samples showing positive KPNA2 staining in at least 10% of nuclei were significantly less frequent in the group of low-grade DCIS (10%) compared to those cases displaying adjacent high-grade DCIS (34.3%). The portion of KPNA2-positive cases in the latter group was almost equivalent to the portion of KPNA2-positive invasive carcinomas, which suggests that KPNA2 overexpression is preceding invasion. Clearly, further studies are needed to clarify the functional role of KPNA2 in breast carcinogenesis.

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